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Prenatal Diagnosis of 30 Fetuses at Risk for Fragile X Syndrome

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The results of 30 prenatal diagnoses for fragile X syndrome are reported. Amniotic fluid cells were examined in 1 case, fetal blood in 4, and chorionic villi samples in the others. Of the 5 fetuses analyzed by cytogenetic methods, 1 had showed 4% of fraXq27.3 expression sites and the pregnancy was terminated. For 1 diagnosis, linkage analysis was used: the female fetus turned out to be normal. In 24 fetuses, the direct analysis of the mutation by StB12.3 probe was performed: 6 female and 3 male fetuses were found to carry a full mutation and 1 female fetus was found to carry a premutation. In 3 cases, the diagnoses were verified on fetal blood samples. Several tissues of 2 aborted male fetuses were analyzed for the fragile X mutation. The results are reported and discussed.

KEY WORDS: prenatal diagnosis, fragile X syndrome

INTRODUCTION

The availability of methods for the direct detection of the FMR1 mutation has made prenatal diagnosis of fragile X syndrome easy to perform, with the results being more informative than those obtained by cytogenetic testing and linkage analysis.

Direct detection is based on the evaluation of the size and methylation pattern of the FMR1 CGG repeats [Kremer et al., 1991; Oberlé et al., 1991; Verkerk et al., 1991]; early prenatal studies showed no differences in the mutation pattern between amniotic fluid cells [Dobkin et al., 1991] and leukocytes, and chorionic villi samples (CVS) presented some unique aspects. In CVS, full mutations may have greater size heterogeneity than in leukocytes: normal methylation related to X inactivation is not clearly evident before the 12th week of gestation, and fragile X full mutation may be not hypermethylated [Hirst et al., 1991; Sutherland et al., 1991; Devys et al., 1992; Suthcliffe et al., 1992].

We report our experience with fragile X prenatal diagnosis, which was performed by cytogenetic analysis before 1989 and then by molecular methods.

MATERIALS AND METHODS

Patients

From 1982 to June 1995, 30 prenatal diagnoses were performed in 28 women (1 had 2 pregnancies and 1 a twin pregnancy). Twenty-seven of the 28 mothers had 1 or more relatives known to be affected by fragile X syndrome; the remaining mother had a family history positive for undiagnosed mental retardation.

In the 6 mothers whose prenatal diagnoses were performed before the StB12.3 probe was available, it was not possible to determine whether they carried a premutation or a full mutation.

Of the 22 women whose carrier status was ascertained by the direct analysis of the mutation, 11 had a premutation and 11 a full mutation.

Fetal Samples

For cytogenetic analysis, amniotic cells were used in 1 case and fetal blood in 4. Molecular analysis was carried out on CVS; in 3 fetuses, the results were not straightforward and, for this reason, were confirmed on blood samples. Constitutional karyotype analysis was performed for all cases.

Cytogenetic Analysis

Constitutional chromosome analysis was performed according to standard techniques; in addition, for the first 5 prenatal diagnoses, the following methods of fragile X induction were used: (1) TC199 + 5% AB serum and (2) TC199 + trimethoprim 15 μg/ml.

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TABLE I. Prenatal Diagnoses Performed by Cytogenetic Analysis

Pedigree i.d.	Tissue	Sex of fetus	fraXq27.3
45/85	Amniotic fluid cells	Male	Negative
332/86	Fetal blood	Male	Negative
45/87	Fetal blood	Male	Negative
260/88	Fetal blood	Female	Negative
172/89	Fetal blood	Male	4%

Molecular Analysis

The CVS DNA was obtained by phenol chlorophorm extraction. Seven micrograms were digested with EcoRI/EagI and with BglII to get sharper smears in cases of faint full mutations. Blotting and hybridization with the probe StB12.3 (kindly provided by Dr. J.L. Mandel) were carried out according to Rousseau et al. [1991]. In the only diagnosis performed by linkage analysis, a number of polymorphic sites flanking the FRAXA locus were investigated.

RESULTS

All DNA and cytogenetic results are reported in chronological order in Tables I and II.

From 1982 to 1989, fraXq27.3 site induction was used for 5 prenatal diagnoses; 1 male fetus showed 4% of fraXq27.3 expression, and the pregnancy was terminated (Table I).

The 25 prenatal diagnoses performed by molecular methods are listed in Table II.

In case 1, PRS91, haplotype analysis demonstrated that the female fetus had inherited the normal X chromosome from the mother.

Twelve fetuses of 11 premutated women were investigated: 8 were normal (5 female and 3 male), 3 were carriers of a full mutation (1 female and 2 male), and 1 twin female had a premutation.

Among the 12 fetuses of the 11 fully mutated mothers, 6 were normal (5 female and 1 male) and 6 carried a full mutation (5 female and 1 male).

In 3 cases, it was necessary to check the fetal blood results. In case 9, BSL94, we considered it necessary to verify the methylation status of the full mutation on fetal blood because the analysis on CVS of the male fetus showed an unmethylated full mutation ($\Delta > 800$ bp). DNA analysis, performed at the 23rd week of pregnancy, showed a methylated full mutation.

We also faced problems with the twin pregnancy because, on CVS analysis, 1 of the 2 female fetuses had normal results and 1 was premutated. Because chorionic villi are not fetal tissues, we wanted to rule out the possibility of mosaicism in the premutated fetus. Therefore, we examined the DNA from blood leucocytes of both fetuses, and the diagnoses were confirmed.

All but 2 of the pregnancies where a fetus carried a full mutation were terminated. One fragile X normal pregnancy was terminated because of trisomy 21.

Five pregnancies are still ongoing; follow-up blood samples were obtained on 11 cases predicted to be normal and on 5 predicted to have full mutations. All predictions were confirmed.

TABLE II. Prenatal Diagnoses Performed by Molecular Analysis[a]

Case	Pedigree i.d.	Maternal mutation	CVS (gestational age)	Fetal blood	Sex of fetus	RFLPs	StB12.3
1	PRS (91)	—	14	—	Female	Normal	—
2	1PGG (92)	Full mutation	11	—	Female	N.I.[c]	Normal
3	**GRM (92)**	**Full mutation**	**11**	**—**	**Female**	**N.I.**	**Full mutation**
4	FNL (92)	Premutation	18	—	Female	—	Normal
5	MNT (93)	Full mutation	10	—	Female	—	Full mutation
6	RND (93)	Premutation	10	—	Female	—	Normal
7	2PGG (94)	Full mutation	11	—	Female	—	Normal
8	FLR (94)	Premutation	11	—	Female	—	Normal
9	**BSL (94)**	**Premutation**	**18**	**Yes**	**Male**	**—**	**Full mutation**
10	**GRP (94)**	**Full mutation**	**13**	**—**	**Female**	**—**	**Full mutation**
11	DLC (94)	Full mutation	11	—	Female	—	Normal
12	PNT (94)	Full mutation	11	—	Male	—	Normal
13	**BDN (94)**	**Full mutation**	**12**	**—**	**Male**	**—**	**Full mutation**
14	PLB (94)	Premutation	12	—	Male	—	Normal
15	LMI (94)	Premutation	14	—	Female	—	Normal
16	**PNU (95)**	**Full mutation**	**11**	**—**	**Female**	**—**	**Normal[b]**
17	BGN (95)	Premutation	12	—	Male	—	Normal
18	RCL (95)	Full mutation	14	—	Female	—	Normal
19	**FRT (95)**	**Premutation**	**12**	**—**	**Male**	**—**	**Full mutation**
20	BDV (95)	Premutation	11	—	Male	—	Normal
21	**RST (95)**	**Full mutation**	**12**	**—**	**Female**	**—**	**Full mutation**
22	**BVN (95)**	**Full mutation**	**12**	**—**	**Female**	**—**	**Full mutation**
23	IPL (95)	Premutation	13	—	Female	—	Full mutation
24	**MMN (95)**	**Premutation**	**10**	**Yes**	**Female**	**—**	**Normal**
25				Yes	Female		Premutation

[a]Terminated pregnancies are indicated by boldface type.
[b]Pregnancy terminated because of trisomy 21.
[c]NI = not informative.

Of the 2 fully mutated male fetuses, BSL94 and BDN94, it was possible to examine a number of tissues: brain, liver, skin, heart, spleen, kidney, lung, testicles, cartilage, and muscle. The 2 fetuses were of different gestational ages: BSL was 25 weeks and BDN was 15 weeks. BSL's mother was premutated and BDN's was fully mutated. The results are shown in Figs. 1 and 2: in BDN, a mosaic premutation/full mutation was present in all the tissues examined (Fig. 1), and a premutated allele was clearly seen only in the lung of fetus BSL (data not shown). In the cartilage and voluntary muscle of both fetuses, smaller bands of 2.8 kb in BDN and 2.6 kb in BSL (Fig. 2) were also present.

DISCUSSION

We found that CVS are the tissue of preference for fragile X prenatal diagnosis because of the speed with which the results are obtained. We had no problems due to maternal contamination, and in all but 3 cases, results on CVS were clear enough to not need fetal blood confirmation. BglII digestion provides better resolution because it narrows the faint full mutation smears. Analysis of the tissues of the aborted fully mutated fetuses can provide information about the mechanisms of amplification, especially when the fetuses are at different gestational ages, as with our 2 cases.

Mosaic premutation/full mutation was demonstrated in all examined tissues from a 15-week male fetus whose mother carried a full mutation. One hypothesis to explain this finding is that the maternal gamete carried a premutation and that the amplification was a postzygotic event [Reyniers et al., 1993].

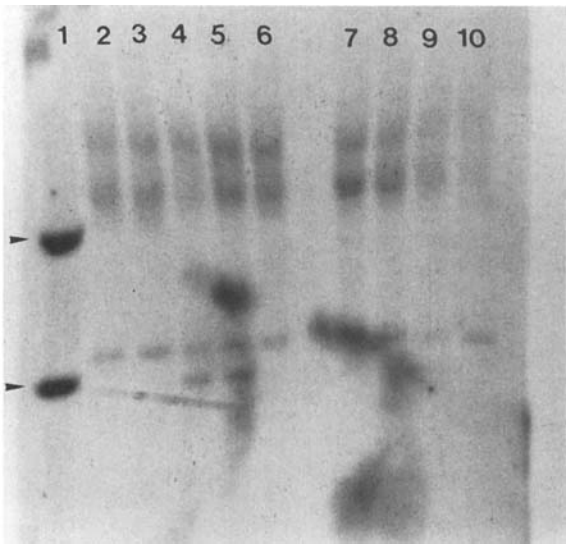

Fig. 1. StB12.3 hybridization pattern of EcoRI/EagI double-digested DNA samples derived from BDN fetal tissues (15 gestational weeks). **Lane 1:** Lung of a normal female fetus. **Lane 2:** Brain. **Lane 3:** Liver. **Lane 4:** Voluntary muscle. **Lane 5:** Cartilage. **Lane 6:** Testicle. **Lane 7:** Heart. **Lane 8:** Lung. **Lane 9:** Spleen. **Lane 10:** Skin.

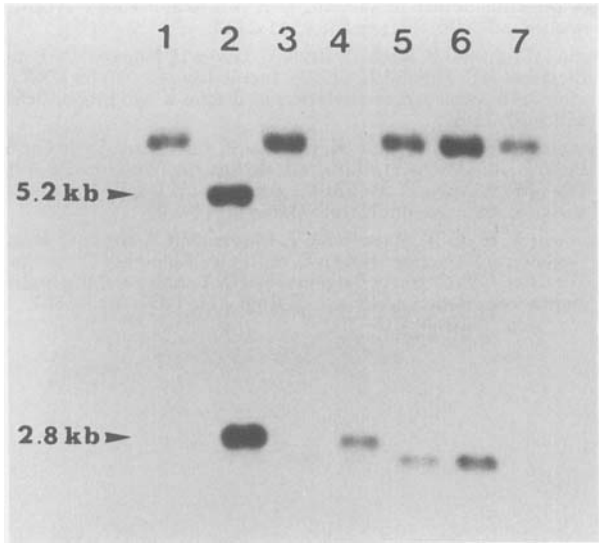

Fig. 2. StB12.3 hybridization pattern of EcoRI/EagI double-digested DNA samples derived from BSL fetal tissues (25 gestational weeks). **Lane 1:** Brain. **Lane 2:** Brain of a normal female fetus. **Lane 3:** Liver. **Lane 4:** Liver of a normal male fetus. **Lane 5:** Voluntary muscle. **Lane 6:** Cartilage. **Lane 7:** Testicle.

The 25-week male fetus whose mother carried a premutation showed a clear pre/full mutation mosaicism only in the lung; presumably, the premutated sequence had already evolved into a full mutation in the other tissues.

It remains to be explained why a smaller allele was clearly present (2.8 kb in 1 fetus and 2.6 in the other) in the cartilage and muscle of both fetuses. The instability of the CGG repeats might be the mechanism that leads to regressions, but whether or not a regression in size in the same tissues of the 2 fetuses is due to chance remains to be clarified.

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